And on page 4, lines 37-38 continuing on to page 5, line 1:

> "The ends of the dsRNA can be modified to counteract degradation in the cell or dissociation into the single strands."

And on page 5, lines 7-10:

> "To inhibit dissociation in a particularly effective fashion, the cohesion of the complementary region II, which is caused by the nucleotide pairs, can be increased by at least one, preferably two, further chemical linkage(s). - A dsRNA according to the invention whose dissociation is reduced exhibits greater stability to enzymatic and chemical degradation in the cell or in the organism."

And on page 5, lines 15-18:

> "To afford protection from degradation, it is expedient for the nucleotides to be chemically modified in the loop region between the doublestranded structure."

Claims 240 and 246 refer to "wherein said dsRNA comprises a 3' overhang" and claims 241 and 247 refer to "wherein said 3' overhang is a single nucleotide overhang". Example 1 on page 9-16 of the above referenced patent application relates to RNA interference in an in vitro transcription assay (human Hela cell nuclear extract). Support for a dsRNA with a 3' overhang and a 3' overhang with a single nucleotide is found in the following citations of the above-referenced patent application:

According to the specification, dsRNA is synthesized from a plasmid, depicted in Figure 1 of the above-referenced patent application, which was constructed as outlined on page 10, lines 1 – 12 of the specification:

> "The plasmid shown in fig. 1 was constructed for use in the enzymatic synthesis of the dsRNA. To this end, a polymerase chain reaction (PCR) with the " positive control DNA" of the HelaScribe ® Nuclear Extract in vitro transcription kit by Promega, Madison, USA, as DNA template was first carried out. One of the primers used contained the sequence of an EcoRI cleavage site and of the T7 RNA polymerase promoter as shown in sequence listing No. 1. The other primer contained the sequence of a BamHI cleavage site and of the SP6 RNA polymerase promoter as shown in sequence listing No. 2. In addition, the two primers had, at the 3' ends, regions which were identical with or complementary to the DNA template."

and on page 10, lines 31 – 38:

> "The PCR product was purified, hydrolyzed with EcoRI and BamHI and, after repurification, employed in the ligation together with a pUC18 vector which had also been hydrolyzed by EcoRI and BamHI. E. coli XL1- blue was then transformed. The plasmid obtained (pCMV5) carries a DNA fragment whose 5' end is flanked by the T7 promoter and whose 3' end is flanked by the SP6 promoter."

Sequence ID No. 1 is disclosed on page 1 of the sequence listing:

> "ggaattctaa tacgactcac tatagggcga tcagatctct agaag"

Sequence ID No. 2 is disclosed on page 2 of the sequence listing:

> "gggatccact taggtgacac tatagaatac ccatgatcgc gtagtcgata"

RNA synthesis was achieved as stated on page 11, line 29 of the specification:

> "pCMV5 plasmid DNA was linearized with EcoRI or BamHI. It was used as DNA template for an in-vitro transcription of the complementary RNA single strands with SP6 and T7 RNA polymerase, respectively."

DsRNA was then generated as stated on page 12, lines 19-30 of the specification:

> "Only approximately equimolar amounts of the two single strands were employed in the hybridization. This is why the dsRNA preparations contained single- stranded RNA (ssRNA) as contaminant. In order to remove these ssRNA contaminants, the reactions were treated, after hybridization, with the single- strand- specific ribonucleases bovine pancreatic RNase A and Aspergillus oryzae RNase T1. RNase A is an endoribonuclease which is specific for pyrimidines. RNase T1 is an endoribonuclease which preferentially cleaves at the 3' side of guanosines. dsRNA is no substrate for these ribonucleases."

For ease of following the above description, we provide the above procedure in schematic terms:

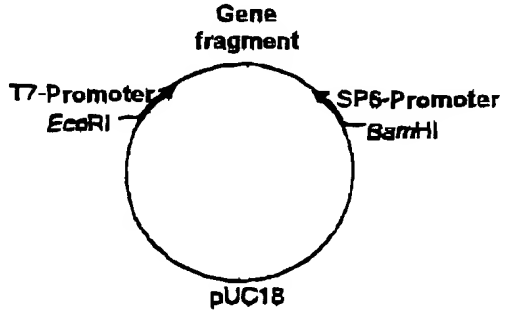
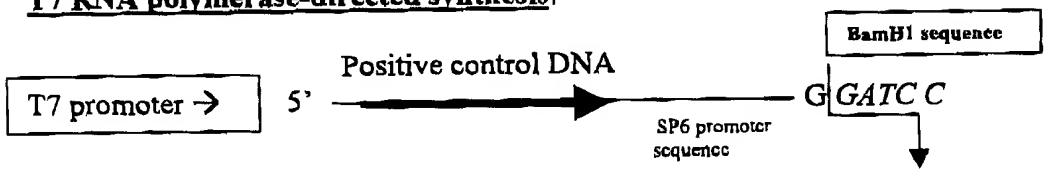


Figure 1 f above-referenced patent application

Attorney Docket N : 20200/2092 (Serial No.:09/889,802)
Inventor: Kreutzer, et al.
Filed: September 17, 2001
Fourth Preliminary Amendment

Linearization of the pCMV5 template with BamH1 or EcoR1:

T7 RNA polymerase-directed synthesis:

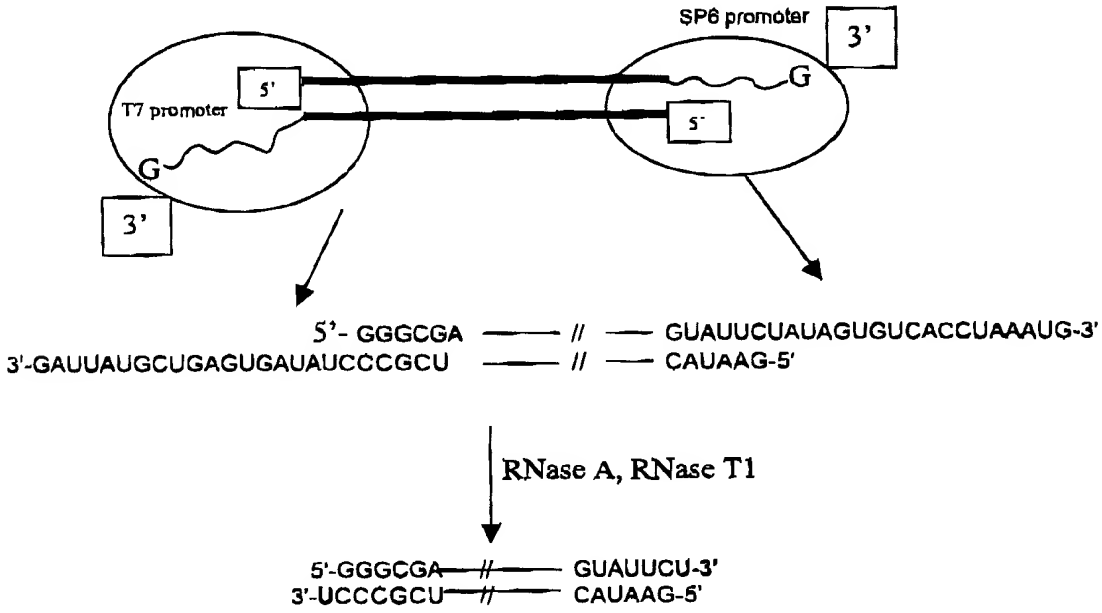


SP6 RNA polymerase-directed synthesis:

ANNEALING OF SP6 AND T7 TRANSCRIPTS:

The sequence of the dsRNA prior to and after RNase digestion, as disclosed in the specification of the above referenced patent application, i.e., in SEQ ID Nos 1 and 2, are depicted in Appendix A. A summary of the sequences pertinent to the generation of a dsRNA with a 3' overhang are depicted below:

DsRNA prior to RNAse digestion:



Attorney Docket No: 20200/2092 (Serial No.:09/889,802)
Inventor: Kreutzer, et al.
Filed: September 17, 2001
Fourth Preliminary Amendment

The site of transcription initiation by SP6 and T7 RNA polymerases was well known in the art at the time of the filing of the above referenced application (see review by Kochetkov, S. et al. FEBS Letters (1998) Pages 440, 264-267 and diagram below).

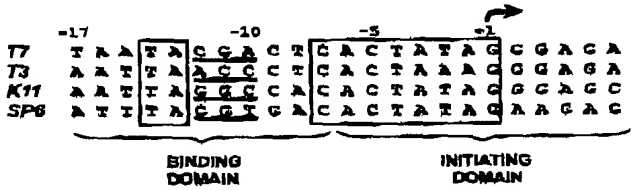


Fig. 2. Consensus sequences of class III promoters of bacteriophages T7, T3, K11, and SP6. The identical sites are boxed. The 'specificity triplets' (see text) are underlined.

SP6 and T7 RNA polymerase promoter sequences (from Kochetkov et al. (1998) *supra*).

The sequence specificity of RNase A and T1 is taught in the specification on page 12, lines 26-29:

> "RNase A is an endoribonuclease which is specific for pyrimidines. RNase T1 is an endoribonuclease which preferentially cleaves at the 3' side of guanosines."

In addition to the teaching in the specification, the cleavage specificity of ribonuclease A was well known in the art at the time of the filing of the above referenced patent application. In Ausubel et. al. Short Protocols in Molecular Biology (1995) 3rd Ed. John Wiley & Sons, Inc., it states on page 3-32:

> "Ribonuclease A (RNase A) from bovine pancreas is an endoribonuclease that specifically hydrolyzes RNA after C and U residues. Cleavage occurs between the 3'-phosphate group of a pyrimidine ribonucleotide and the 5'-hydroxyl of the adjacent nucleotide."

It was therefore known in the art as of the Applicant's filing date that an RNase resistant double stranded RNA generated by RNase digestion as disclosed in the above referenced patent application would have a 3'overhang of one nucleotide.

Claim 242 refers to an oligoribonucleotide according to claim 221 which is 21 or 23 nucleotides in length. Support for this claim is found at page 17, lines 18-21 of the above-referenced patent application:

> "A dsRNA linked chemically at the 3' end of the RNA as shown in sequence listing No. 8 to the 5' end of the complementary RNA via a C18 linker group was prepared (L-dsRNA)."

And on page 4 of the sequence listing:

> "Description of the artificial sequence: RNA which corresponds to a sequence from the YFP gene.
>
> C400> 8
> ucgagcugga eggcgacpua a 21."

And page 11, lines 7-8:

> "In accordance with the method outlined hereinabove, an RNA 23 nucleotides in length was also synthesized."

Claim 243 refers to "a composition comprising an oligoribonucleotide according to claim 221. Support for this claim is found at page 7, lines 26-31 of the above-referenced patent application:

> "The invention furthermore provides a medicament with at least one oligoribonucleotide with double- stranded structure (dsRNA) for inhibiting the expression of a given target gene."

And page 14, lines 17-18:

> "various amounts of dsRNA in transcription buffer were employed per reaction."

And on page 18, lines 27 – 32:

> "The samples injected into the nuclei contained 0.01 μ g/ μ l of pCDNA- YFP and Texas Red coupled to dextran- 70000 in 14 mM NaCl, 3 mM KCl, 10 mM KPO4 [sic], ph 7.5. Approximately 100 pl of RNA with a concentration of 1 μ M or, in the case of the L-dsRNA, 375 μ M were additionally added."

Claim 244 refers to a composition which includes a first oligoribonucleotide according to claim 221 and a second oligoribonucleotide according to claim 221, wherein the two sequences

differ. Support for this claim is found at page 7, lines 13-17 of the above-referenced patent application:

> "At least two dsRNAs which differ from each other or at least one vector encoding them can be introduced into the cell, where at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes."

In view of the extensive support present in the above-referenced patent application, as outlined above, it is submitted that the newly added dependent claims are clearly described, defined and supported in the instant patent application. Applicants submit that no new fees are believed to be required for newly added claims 239-246, but hereby authorize the Commissioner to charge any additional claim fees to Deposit Account No. 16-0085, Reference No. 20200/2092.

Applicants respectfully request entry of the above amendments.

Respectfully submitted,

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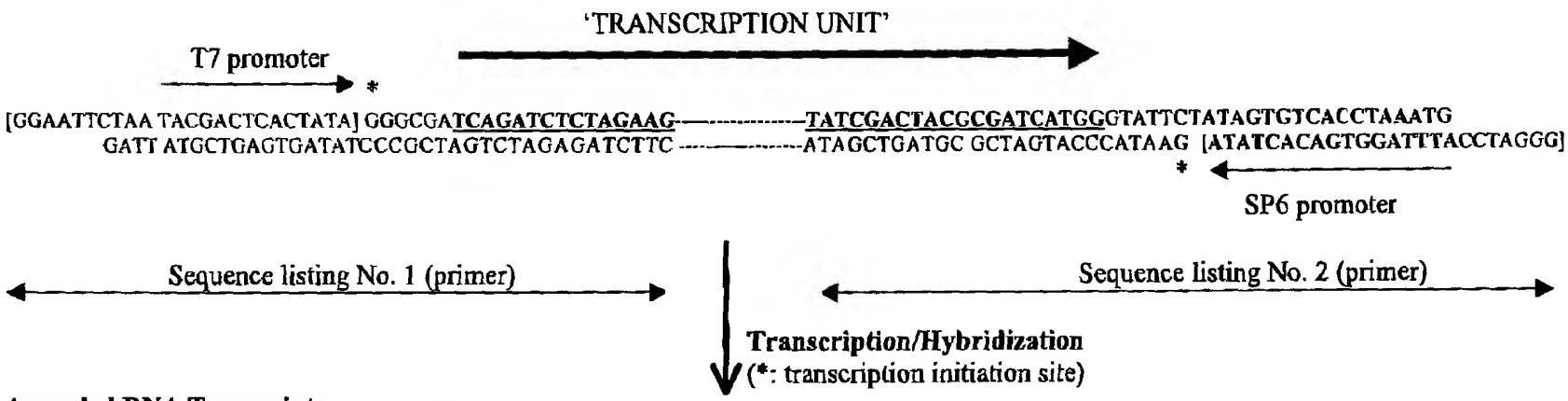
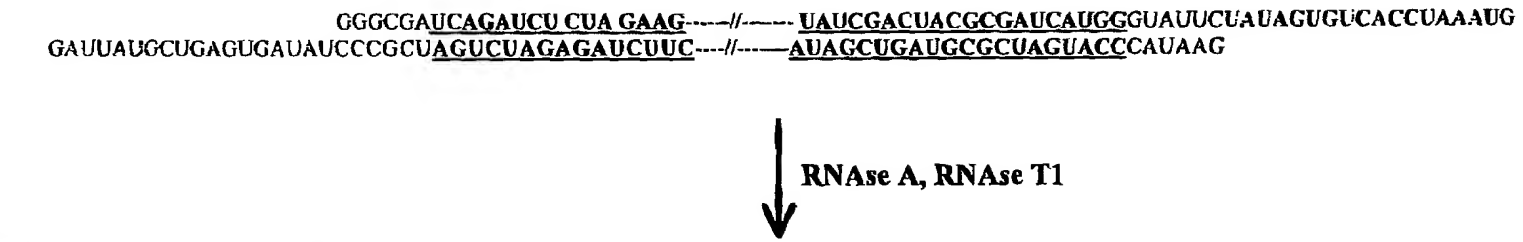
APPENDIX A:

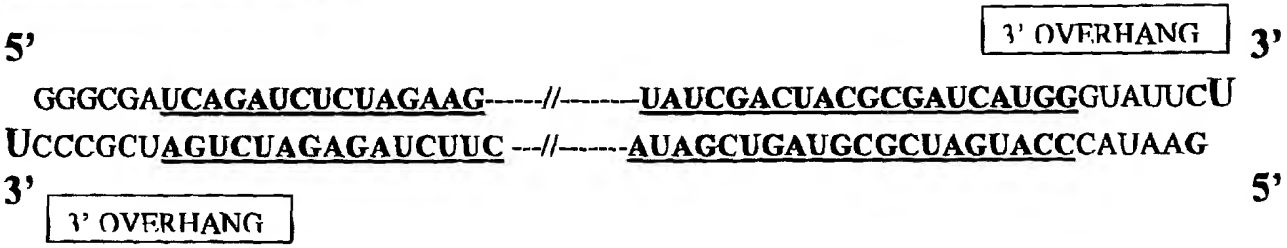
pCMV5 DNA Template:



Annealed RNA Transcripts:



RNAse resistant dsRNA:



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03/07/03 FRI 15:02 [TX/RX NO 8851] 010 ** TOTAL PAGE.10 **